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Epidemiological Analysis of Multi-Site Closure Failure of Neural Tube in Humans

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Van Allen et al. [Am J Med Genet 47:723–743, 1993] proposed that there is multi-site initiation of neural tube closure in humans, and that neural tube defects (NTD) represent the failure of one or more of 5 closure sites.

We have studied from an epidemiologic perspective 774 liveborn infants with NTD by site of lesion following the multi-site classification proposed by Van Allen et al. [1993]. As predicted by these authors, we could classify all the cases with NTD by the multi-site closure model. We have also estimated the prevalence of each failure closure site. This analysis indicates that not all the sites are affected with similar frequency.

KEY WORDS: multi-site NTD, epidemiology, frequencies

INTRODUCTION

Van Allen et al. [1993] proposed that there is a multi-site initiation of neural tube closure in humans similar to that observed in experimental animals. These authors consider neural tube defects (NTD) as representing failure of one or more of 5 closure sites. We have analyzed from epidemiologic perspectives 774 liveborn infants with NTD, classified according to the multi-site model proposed by Van Allen et al. [1993].

MATERIALS AND METHODS

The study was based on 774 liveborn malformed infants with neural tube defects (NTD) detected during the first 3 days of life. The malformed infants were identified through the Spanish Collaborative Study of Congenital Malformations (ECEMC). The methodology of this hospital-based case-control study and surveillance system is aimed not only at the surveillance of congenital anomalies, but also at investigating their characteristics, clustering, and causes. The methodology was published previously [Martínez-Frías et al., 1991; Martínez-Frías, 1994, 1995].

As reported previously [Martínez-Frías, 1994; Martínez Frías and Urioste, 1994] the ECEMC coding system has different levels to identify clinical patterns. This allows retrieval, as isolated cases, of clinical patterns of those infants with only one defect including its sequence. For instance, we have also considered as isolated spina bifida those infants with hydrocephaly, club foot, congenital hip dislocation, etc. For the present analysis we have reviewed the clinical description of each infant with NTD as well as photographs and/or complementary studies if available, in order to classify them according to the closures 1–5 in humans as proposed by Van Allen et al. [1993]. To do this, we have expanded the ECEMC code for each NTD in order to specify the location of the lesion according to the following closure sites 1–5: closure 1 occurs in the midcervical region and progresses bidirectionally in the rostral and caudal direction. Closure 2 begins in the head at the junction between the prosencephalon (forebrain) and mesencephalon (midbrain), forming two neuropores and proceeds bidirectionally. In the rostral direction it proceeds over the prosencephalon to meet closure 3. In the caudal direction it progresses over the mesencephalon to terminate at the superior part of the rhombencephalon. Closure 3 is unidirectional and appears from the stomodeum and goes caudally to meet the descending rostral segment of closure 2. The site of the junction of closures 2 and 3 corresponds to the interorbital region of the face. Closure 4 begins at the caudal end of the rhombencephalon and is unidirectional, proceeds rostrally to meet the caudal aspect of closure 2. Closure 5 is initiated at the most caudal end of the neural tube and proceeds rostrally to meet the caudal 1 closure. It affects the area of the posterior neuropore in the lumbar and sacral region [Golden and Chernoff, 1993; Van Allen et al., 1993].

For the present analysis we separated the cases into two time periods: one from April 1976 to 1985, during

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which voluntary termination of pregnancy (VTOP) was not legal in Spain, and from 1986 to 1994, during which the VTOP due to malformations in the embryo was permitted by law.

RESULTS

Table I presents the study population separating the cases with NTD by the number of affected sites in the closure defect of neural tube (NT). In many cases it was not possible to be sure about the location of the lesion and, although the description or photographs strongly supported a particular type, these cases have been included as suspected. As Table I indicates, if we accept the suspected cases as true, the most frequent type of neural tube closure defect is that affecting two contiguous sites (more than 50% in the two studied periods), followed by the defect of only one site. It is also interesting that about 1.5% of the cases involved more than one noncontiguous site. The differences in the proportion of different closure site failure between the two studied periods could well be due to a better description of the cases during the second studied period. In the same table the prevalence figures are presented. The differences in the prevalence figures observed between the two studied periods could be explained as being produced by the prenatal diagnosis followed by the VTOP in the second period.

Table II depicts the prevalence figures and the proportion of cases with each closure failure site. In this Table, if a child has more than one affected site it is included in the different affected sites. When the defect is located between two contiguous sites, i.e., if one case has the site 1 + 4 affected, the infant is included in the group with site 1 affected and in the group with site 4 affected. The observed differences in the proportions of each affected closure site between the two studied time periods could be due to a better description of cases in the second period, as well as by differences in the impact of the VTOP in each affected site. Table II shows that among the cases of the first period (without VTOP), the prevalence of closure failure is lower for sites 3 and 5, while sites 1, 2 and 4 have a higher prevalence and are affected with similar frequency. The differences with the figures of the second period are probably due to the VTOP. Nevertheless, the increased prevalence of closure failure 5 observed in the second period could be random since the difference from the first period is not statistically significant. However, we cannot totally exclude a potential slight increase of the prevalence due to unknown causes.

As there is an important group of cases in which the exact closure failure sites are "suspected," in the following tables we include each type of closure failure separating those clearly specified from those which are suspected. Table III distributes the cases by type of failure closure site(s) of neural tube. In the first time period (when the VTOP was not permitted), among the cases with clear site involvement, the most frequent is the closure failure of only site 1 (2.07 per 10,000 liveborn infants). But if we accept the suspected cases as truly affected, then the most frequent failure of NT closure is that affecting contiguous sites 2 and 4 (2.65 per 10,000) even in liveborn infants. The differences between the first and second time periods are probably due to the prenatal diagnosis and subsequent VTOP.

Table IV distributes the cases by clinical presentation. Table V presents the distribution of cases by cause. We have considered as multifactorial all isolated cases with the different NTD. The most frequent lesion of genetic origin affects site 4.

In 20 (2.58%) out of 774 cases with NTD there were affected sibs. Table VI shows the closure failure site in each affected case and their sibs.

DISCUSSION

We have studied the frequency of the different NTD by site of lesion following the multi-site classification proposed by Van Allen et al. [1993], among livebirths. As predicted by these authors, we could classify all the cases with NTD by the multi-site closure model. The difficulty observed in some cases was only due to the lack of information and/or an adequate description. The analysis of the proportions of the different types of lesion concurs with the observation of Van Allen et al. [1993] among their clinical cases.

As far as we know, there is only one previous report [Moore et al., 1995] on the prevalence of the different sites of closure failure of NT in two populations from

TABLE I. Frequency of the Lesion by Number of Affected Sites and in Two Time Periods

	1976–1985			1986–1994		
Failed closure site(s)	N	%	Per 10,000 (TLB[a] = 550,256)	N	%	Per 10,000 (TLB = 650,245)
Affecting only one site	166	37.64	3.02	141	42.34	2.17
Affecting two contiguous sites	25	5.67	0.45	32	9.61	0.49
Suspected that it affects two contiguous sites	214	48.53	3.89	141	42.34	2.17
Affecting three contiguous sites	1	0.23	0.02	0	—	—
Suspected that it affects three contiguous sites	5	1.13	0.09	0	—	—
Affecting more than one noncontiguous site	7	1.59	0.13	5	1.50	0.08
Not specified	23	5.22	0.42	14	4.20	0.22
Total infants with NTD	441	100	8.01	333	100	5.12

[a] TLB, total liveborn infants.

TABLE II. Prevalence Figures and Proportion of Infants by Each of the Affected Closure Sites and in Two Time Periods

	1976–1985			1986–1994		
Failed closure site(s)	N	%	Per 10,000 (TLB = 550,256)	N	%	Per 10,000 (TLB = 650,245)
Affecting site 1	210	47.62	3.82	230	69.07	3.54
Affecting site 2	174	39.46	3.16	56	16.82	0.86
Affecting site 3	14	3.17	0.25	5	1.50	0.08
Affecting site 4	187	42.40	3.40	75	22.52	1.15
Affecting site 5	94	21.32	1.71	135	40.54	2.08
Total infants with NTD	441	100	8.01	333	100	5.12

China, one with high prevalence and another with low prevalence of NTDs. Among the 338 infants with NTD analyzed by those authors, there were no cases with closure failure of site 3. The method of grouping the different sites of closure failure followed by Moore et al. [1995] is different from that of the present analysis, because these authors separated the closure failure 1 into different levels. Thus, the comparison of the prevalence figures of the two studies is difficult. Nevertheless, their prevalences seem to be higher than ours.

In our data, the closure failures of sites 2, 4, and 1 are the most frequent and have similar prevalence figures among liveborn infants, site 3 being less frequent. This location of the lesion is extremely rare. In fact, among the 19 cases we have observed (in both time periods) with closure failure 3 (Table II), 3 were orbital encephaloceles, 8 frontal encephaloceles, 6 were faciocranioschisis (closure failures in sites 3 + 2 + 4), and 2 had only closure failure 3. One of these 2 cases had hydrocephaly, central cleft lip and cavum pharyngis encephalocele. The other case was an infant with only schisis of site 3 (facioschisis) without craniorachischisis and without other anomalies. This case is going to be reported separately as apparently the first case with this rare type of NTD closure.

Although the study was done on liveborn infants, among the total of 161 stillborn infants with NTD from the period 1980–1994 of the ECEMC data, sites 2 and 2 + 4 were the most frequently affected sites (73.29%), while site 1 was only affected in the 14.91% of the total stillborn cases. The noncontiguous NTDs represented the 2.48% of the total stillborn cases. Their distribution by clinical presentation and causal agents was similar to that observed in liveborn cases.

In about 50% of the cases, the NTD affects or implies two contiguous closure sites. This suggests that the junction domain of 2 sites constitutes a weak area and, consequently, could have higher frequency of closure failure.

We have observed a total of 12 cases with noncontiguous NTDs. Among the 441 studied cases in the first period of time, 1.59% presented noncontiguous NTDs, representing a prevalence of 0.13 per 10,000 liveborn infants. Reynolds et al. [1995] studying 13 cases with noncontiguous NTDs stated that these cases cannot be

TABLE III. Frequency of the Different Site Lesions of Neural Tube Defects in Two Time Periods

	1976–1985 (TLB = 550,256)		1986–1994 (TLB = 650,245)	
Failed closure site(s)	N	Per 10,000	N	Per 10,000
Affecting only site 1	114	2.07	93	1.43
Affecting only site 2	13	0.24	12	0.18
Affecting only site 3	1	0.02	1	0.02
Affecting only site 4	34	0.62	32	0.49
Affecting only site 5	4	0.07	3	0.05
Affecting sites 1 + (4 suspected)	0	—	1	0.02
Affecting sites 4 + (1 suspected)	0	—	1	0.02
Affecting sites 1 + 5	10	0.18	21	0.32
Affecting sites 1 + (5 suspected)	79	1.44	109	1.68
Affecting sites 3 + 2	4	0.07	2	0.03
Affecting sites 2 + 4	11	0.20	9	0.14
Affecting sites 2 + (4 suspected)	135	2.45	30	0.46
Affecting sites 2 + 4 + 1	1	0.02	0	—
Affecting sites 3 + 2 + (4 suspected)	4	0.07	0	—
Affecting sites 2 + 4 + (3 suspected)	1	0.02	0	—
Discontinued sites 1 and 3 + 2	4	0.07	2	0.03
Discontinued sites 1 and 4	1	0.02	1	0.02
Discontinued sites 1 and 1 + 5	0	—	1	0.02
Discontinued sites 2 and 1 + 5	1	0.02	0	—
Discontinued not clearly described	1	0.02	0	—
Discontinued 4 + 1, 2 + 4, 1 + (5 suspected)	0	—	1	0.02
Sites not specified	23	0.42	14	0.23
Total infants	441	8.01	333	5.12

TABLE IV. Distribution of Cases by Site of the Lesion and Clinical Presentation

Failed closure site(s)	Isolated	With MCA pattern	Syndromes	Total
Affecting only site 1	164	31	12	207
Affecting only site 2	23	1	1	25
Affecting only site 3	1	1	0	2
Affecting only site 4	32	17	17	66
Affecting only site 5	7	0	0	7
Affecting sites 1 + (4 suspected)	0	1	0	1
Affecting sites 4 + (1 suspected)	1	0	0	1
Affecting sites 1 + 5	23	8	0	31
Affecting sites 1 + (5 suspected)	157	24	7	188
Affecting sites 3 + 2	2	4	0	6
Affecting sites 2 + 4	14	5	1	20
Affecting sites 2 + (4 suspected)	159	6	0	165
Affecting sites 2 + 4 + 1	1	0	0	1
Affecting sites 3 + 2 + (4 suspected)	1	3	0	4
Affecting sites 2 + 4 + (3 suspected)	1	0	0	1
Discontinued sites 1 and 2 + 3	3	1	2	6
Discontinued sites 1 and 4	2	0	0	2
Discontinued sites 1 and 1 + 5	0	1	0	1
Discontinued sites 2 and 1 + 5	1	0	0	1
Discontinued not clearly described	1	0	0	1
Discontinued 1 + 4, 2 + 4, 1 + (5 suspected)	0	0	1	1
Sites not specified	30	4	3	37
Total infants	623	107	44	774

explained on the basis of the model of a single initiation site with bi-directional closure. On the contrary, they suggested that those cases support the multi-site model of neural tube closure proposed by Van Allen et al. [1993].

All sites of NT closure failure are observed as isolated cases, as cases with multiple congenital anomaly (MCA) patterns and in different syndromes. Similarly, most of the types of failure closure are causally heterogeneous. However, although many cases with each site of closure failure are multifactorial, NTD site 4 is the most frequent among those of genetic cause (Table IV), while the failure in site 1 is the most frequent among those of chromosomal or environmental cause (Table V). However, as shown by Seller [1995], a particular agent, such as folate, could have the same effect on all the NTD types. Nevertheless, the possible differential effect of other agents should be investigated.

In conclusion, based on our epidemiological data, we agree with Van Allen et al. [1993] that all NTDs "can be

TABLE V. Distribution of Cases by Site of the Lesion and Etiology

Failed closure site(s)	Multifactorial	Recessive	Chromosomal	Unknown cause	Environmental
Affecting only site 1	154	0	3	41	9
Affecting only site 2	19	0	1	5	0
Affecting only site 3	1	0	0	1	0
Affecting only site 4	28	12	0	23	3
Affecting only site 5	4	0	0	3	0
Affecting sites 1 + (4 suspected)	0	0	0	1	0
Affecting sites 4 + (1 suspected)	1	0	0	0	0
Affecting sites 1 + 5	22	0	0	9	0
Affecting sites 1 + (5 suspected)	148	0	2	33	5
Affecting sites 3 + 2	1	0	0	5	0
Affecting sites 2 + 4	10	1	0	9	0
Affecting sites 2 + (4 suspected)	147	0	0	18	0
Affecting sites 2 + 4 + 1	1	0	0	0	0
Affecting sites 3 + 2 + (4 suspected)	1	1	0	2	0
Affecting sites 2 + 4 + (3 suspected)	1	0	0	0	0
Discontinued sites 1 and 3 + 2	2	0	2	2	0
Discontinued sites 1 and 4	2	0	0	0	0
Discontinued sites 1 and 1 + 5	0	0	0	1	0
Discontinued sites 2 and 1 + 5	1	0	0	0	0
Discontinued not clearly described	1	0	0	0	0
Discontinued 1 + 4, 2 + 4, 1 + (5 suspected)	0	0	0	0	1
Sites not specified	23	2	1	11	0
Total infants	567	16	9	164	18

TABLE VI. Infant With NTD With Affected Sibs by Site of Lesion

Closure failure site of the affected infant (case)	Closure failure site of the affected sib(s)
1 + (5 suspected)	1 + (5 suspected)
1	1 + (5 suspected) co-twin[b]
4	Meningocele NS[c]
4 (Meckel S.[a])	4 (Meckel S.)
4	Spina bifida NS
	2 + (4 suspected)
	Encephalocele NS
	4
2 + (4 suspected)	2 + (4 suspected)
1 + (5 suspected)	Myelomeningocele NS
4	4
1 + (5 suspected)	1
1	Myelomeningocele NS
1	Meningocele NS
2 + 3 + (4 suspected)	2 + 3 + (4 suspected)
	2 + 3 + (4 suspected)
1 + (5 suspected)	Myelomeningocele NS
2 + (4 suspected)	Spina bifida NS
2 + (4 suspected)	2 + (4 suspected)
4 (Meckel S.)	Encephalocele NS (Meckel S.)
1 + (5 suspected)	1 + (5 suspected) co-twins[b]
2 + (4 suspected)	2 + (4 suspected)
1 + (5 suspected)	1 + (5 suspected) co-twins[b]
Encephalocele (Meckel S.)	Meckel syndrome

[a] S., Syndrome.
[b] In the three pairs of twins, both co-twins were concordant in sex.
[c] NS, Not specified.

explained by failure of fusion of one of the closures or their contiguous neuropores." On the other hand, most of the closure site defects are causally heterogeneous. As shown in Table VI, all closure sites except site 3 were observed in sibs and same-sex twins with high concordance, suggesting a genetic compound. These observations are also in agreement with the conclusion of Van Allen et al. [1993] that "closure sites are most likely controlled by separate genes expressed during embryogenesis, and variations in rate and location of closures would make embryos more susceptible to environmental and other factors." The analysis of the prevalences in our population indicates that not all the sites are affected with similar frequency. The study of the prevalences in other populations could allow elucidation of their general characteristics, as well as those related with the make-up of each population.

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